UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

**Support Document #19**

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM OCT 21 1994

SUBJECT: Closure on Nontarget Plant Phytotoxicity Policy Issues

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The Plant Risk Evaluation Team (PRET) had come to closure on a number of nontarget plant phytotoxicity policy issues. The PRET team suggests that these changes be initiated as soon as possible by the branch and division. The PRET believes that these changes are a beginning step forward in our efforts to eliminate confusion and review inconsistencies. These issues are considered Subdivision J/SEP house cleaning items that help to clarify vague and often misleading statements in these documents.

Guideline harmonization, NACA rejection rate concerns, and RD/SRRD nontarget plant risk assessment concerns all point toward the need to expand the scope of nontarget plant phytotoxicity testing.

AQUATIC PLANT GROWTH STUDIES (122-2, 123-2)

1.) Four or 5 day algal studies will be accepted for review by the Agency. Three day OECD studies will be reviewed as Tier I screening studies only. (This is a harmonization issue.)

2.) Seven or 14 day Lemna gibba aquatic macrophyte studies will be accepted for review by the Agency. (This is a harmonization issue.)

3.) For the studies: Selenastrum capricornutum, Anabaena flos-aquae, Skeletonema costatum, or Navicula pelliculosa up to 10,000 cells per ml initial cell concentration are allowed. For Anabaena flos-aquae, cell counts on day 2 are not required.

4.) Sonification during algal tests is not allowed for the entire course of the study (INVALID), except for limited Anabaena flos-aquae sonification as described in 1991 Annual Book of ASTM Standards, Volume 11.04, E1218-90, pgs 845-856, "Standard Guide For Conducting Static 96-h Toxicity Tests with Microalgae".

5.) For the Skeletonema costatum study, the photoperiod should be: 14 hours light/10 hours dark. Continuous light is recommended for the other 4 aquatic plant test species. Light intensity should be approximately 4.3 K lux for Selenastrum capricornutum, Skeletonema costatum, and Navicula pelliculosa; 2.2 K lux for Anabaena flos-aquae; and 4.2-5.8 K lux for Lemna gibba.

6.) Due to erratic growth, Navicula sp. tests should be conducted with 4 replicates.

7.) The lack of a no-effect concentration will not invalidate an acute study as long as the data are adequate for calculation of valid EC50, EC25, and NOEC (or EC05) values from the most sensitive endpoints. Studies lacking a no-effect test concentration but meet the above criteria will be called supplemental.

| IF THE LOWEST TEST LEVEL AND THE NOEC ARE: | | THEN THE STUDY MAY BE: |
|---|---|---|
| < EC10 | - | CORE |
| > EC10, < EC25 | - | SUPPLEMENTAL |
| > EC25 | - | INVALID |

8.) Requests for 3X and 4X geometric dosage progressions (instead of the mandatory 2X in Subdivision J) will be honored with the proviso that the registrants extend the test for the most sensitive species if the no-effect level (or EC05) has not been achieved. The test must define the response curve so that accurate EC50, EC25, and NOEC (or EC05) values can be estimated.

9.) For Lemna gibba, a solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used. Refer to 1991 Annual Book of ASTM Standards, Volume 11.04, pgs. 1137-1146, "Standard Guide For Conducting Static Toxicity Tests With Lemna gibba G3".

10.) For Lemna gibba, the total number of recommended plants per chamber is 3 to 5 with 3 to 5 fronds each. Each chamber must have the same number of plants with an identical number of fronds per plant. Refer to 1991 Annual Book of ASTM

Standards, Volume 11.04, pgs. 1137-1146, "Standard Guide For Conducting Static Toxicity Tests With Lemna gibba G3".

11.) For Skeletonema costatum marine diatom, a salinity of 35 ppt (parts per thousand) is acceptable.

12.) Deviation in light intensity of up to 15% is acceptable for aquatic nontarget plant growth studies.

13.) Test concentrations in the Lemna gibba studies are expected to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).

14.) If solvents or other pesticides are used in the test, the registrant must show that the solvent/pesticide is not toxic to the test species and that no synergistic or antagonistic interactions with the test pesticide exists (additional test data). If the registrant so chooses, they may treat a separate set of control plants (set aside for this purpose at the beginning of the experiment) with the solvent/pesticide using the highest dosage. This would be done for each solvent/pesticide added to the test to evaluate synergism/antagonism. A negative control is still required. No other pesticide treatments are recommended.

15.) If the solvent/pesticide control(s) and the negative control are excessively contaminated with the test chemical (as based on best professional judgement), the study **may** be **INVALID**. If the solvent control is contaminated with the test chemical and the negative control is not (or visa versa), the study may not be invalid if 0% toxicity occurred in the negative control and at the lowest dose tested.

16.) A statistically derived NOEC or EC05 value will serve as the aquatic plant endangered species trigger.

17.) The use of TEP (typical end-use product) instead of TGAI (technical grade active ingredient) is hereby recommended for all aquatic plant growth studies (122-2, 123-2). If an adjuvant(s) is recommended on the product label, a representative adjuvant(s) must be included in the test at the recommended dosage. The TEP selected for testing should be the one with the highest percentage active ingredient and/or the one most commonly used. TEP's that contain other active ingredients should be avoided or tested separately. The use of TEP testing should eliminate the need for a separate solvent control as the solvents will already be contained in the formulation. A negative control is still required.

## TERRESTRIAL NONTARGET PLANT GROWTH STUDIES (122-1, 123-1)

1.) 122-1 and 123-1 seed germination studies **are hereby generally waived** by the agency and are not required to be conducted except on a limited, as needed, case-by-case basis. The 122-1 and 123-1 seedling emergence study must still be conducted. (This is a current CFR40, Part 158 issue).

2.) A statistically derived NOEC or EC05 value will serve as the terrestrial endangered plant species trigger.

3.) For the seedling emergence test, the minimum acceptable USDA seed germination (control) standards (Federal Seed Act Regulation, 7 CFR Part 201-201) are: field corn (85%), pop corn (75%), sweet corn (75%), carrot (55%), onion (70%), tomato (75%), field - garden bean (70%), pea (80%), pepper (55%), beet (65%), buckwheat (60%), cabbage (75%), lettuce (80%), mustard - all types (75%), soybean (75%), sugarbeet (55%), small grains - wheat - oats - barley - rice (80%), ryegrass (75%), vetch (75%), alfalfa - clover (70%), rape (75%). Refer to regulation for other vegetable crops. If available, report the % germination for the seed batch used. This information is usually supplied by the seed suppliers.

4.) Subdivision J is not specific with regard to test plot watering method. The agency prefers that bottom watering be utilized for seedling emergence studies so that the chemical is not leached out of the soil during the test. The registrant should assess the pesticides' potential for leaching as based on solubility and Kd value. For vegetative vigor studies, top watering under the foliage or bottom watering are recommended so that chemical is not washed off the foliage.

5.) Subdivision J does not specify what type of pot/container to use, but the Agency recommends that non-porous containers be used. Peat and clay containers may absorb the test chemical.

6.) Subdivision J does not specify propagation media. Soil mixes containing sandy loam, loam, or clay loam soil with no greater than 2% organic matter are preferred. Glass beads, rockwool, and 100% acid washed sand are not recommended.

7.) Subdivision J does not specify the number of plants per pot/container. Too many plants in one container can lead to overcrowding, especially if the study is conducted beyond 14 days. We will continue to leave pot size to the discretion of the registrant. A guide for a 6 inch pot might be: 1 to 2 corn, soybean, tomato or cucumber plants; a maximum of 3 sugarbeet, rape, or pea plants; a maximum of 6 onion, wheat

or other small grains per pot.

8.) Subdivision J does not specify test temperature. Some of the Subdivision J study plants are cold hardy, and some prefer warmer temperatures. The Agency prefers that the cold vs warm loving plants be tested in 2 separate groups to optimize plant growth.

9.) If solvents or other pesticides are used in the test, the registrant must show that the solvent/pesticide is not toxic to the test species and that no synergistic or antagonistic interactions with the test pesticide exists (additional test data). If the registrant so chooses, they may treat a separate set of control plants (set aside for this purpose at the beginning of the experiment) with the solvent/pesticide using the highest dosage. This would be done for each solvent/pesticide added to the test to evaluate synergism/antagonism. A negative control is still required. **No** other pesticide treatments are recommended.

10.) If the solvent/pesticide control(s) and the negative control are excessively contaminated with the test chemical (as based on best professional judgement), the study **may** be **INVALID**. If the solvent control is contaminated with the test chemical and the negative control is not (or visa versa), the study may not be invalid if 0% toxicity occurred in the negative control and at the lowest dose tested.

11.) Whenever possible, the registrants have agreed to use **untreated seeds** in terrestrial nontarget plant studies (NACA/EPA Study Rejection Rate Meeting, Apr. 1994). Untreated seeds are those that have not been dusted or soaked in insecticides or fungicides. Steam sterilization of untreated seeds is the recommended procedure to kill pathogens, fungi, and insects on seeds and in soil media. A weak clorox solution, as recommended by Canada in their guidelines is also acceptable as a seed treatment. Some methods used to remove seed treatments include weak methanol, detergent, and clorox solution rinsing. When seed such as sugarbeet cannot be grown without a previous seed treatment, the registrant should so state and provide baseline germination data for that seed (sugarbeet in this case) with and without the treatment(s). It is the responsibility of the registrant to show that no synergistic or antagonistic interactions occur between or among the various pesticides in the test. If the registrant is justified in using treated seeds, and there are no other major rejection factors, the study may be called SUPPLEMENTAL and up-gradable to CORE upon submission of additional interaction data. Registrants are encouraged to conduct the necessary research on pesticide interactions. Tier II plant phytotoxicity studies for seed treatments

would also provide useful data regarding direct toxicity to plants.

12.) The use of other pesticide treatments to control pests on plants after plant emergence are not recommended. Herbicide/insecticide interactions are known to exist: organophosphate insecticide use to safen cotton plants from clomazone herbicide injury, sulfonylureas should not be used within 2 weeks of OP insecticide sprays to avoid enhanced herbicide injury. If an unexpected pest outbreak occurs, the registrant should so state. If other pesticides are used in the study, the registrant must show that no synergistic/antagonistic interactions occurred by way of interaction research or the use of (insecticide or fungicide) treated controls. This involves setting aside a number of "control" plants to be treated as needed during the course of the study. If the registrant is justified in using the emergency treatment, and there are no other major rejection factors, the study **may** be called SUPPLEMENTAL and up- gradable to CORE upon submission of additional interaction data. If treated control are used, the study **may** be called CORE.

13.) The use of TEP (typical end-use product) instead of TGAI (technical grade active ingredient) is hereby recommended for all terrestrial nontarget plant tests. If an adjuvant(s) is recommended on the product label, a representative adjuvant(s) must be included in the test at the recommended dosage. The TEP selected for testing should be the one with the highest percentage active ingredient and/or the most widely used. TEP's that contain other pesticide active ingredients should be avoided or tested separately. The use of TEP testing should eliminate the need for a separate solvent control as the solvents will already be contained in the formulation. A negative control is still required. (This is a current CFR40, Part 158 issue).

14.) The registrant is expected to reasonably extend the test period beyond 14 days as dictated by the pesticides mode-of-action. Different pesticides are translocated throughout plants at different speeds. Some of the newer herbicides require 3 to 4 weeks to adversely affect plant growth. While Subdivision J states a 14 day test period for these tests, the registrants and the EPA reviewers must use best judgement regarding the adequacy of the test period. Registrants are encouraged to submit mode-of-action data with these studies.

15.) The lack of a no-effect concentration will not invalidate an acute study as long as the slope is adequate for calculation of valid EC50, EC25, and NOEC (or EC05) values from the most

sensitive endpoints (e.g. dry shoot weight, dry root weight, shoot height). Studies that lack a no- effect test concentration but meet the above criteria will be called SUPPLEMENTAL.

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| < EC10 | - | CORE |
| > EC10, < EC25 | - | SUPPLEMENTAL |
| > EC25 | - | INVALID |

16.) Requests for 3X and 4X geometric dosage progressions (instead of the mandatory 2X in Subdivision J) will be honored with the proviso that the registrant extend the test for the most sensitive species if the no-effect level (or EC05) has not been achieved. The test must define the response curve so that accurate EC50, EC25, and NOEC values can be estimated.

17.) Subdivision J endpoints include % seedling emergence, severity of phytotoxicity (observed), and suggests direct measurements of adverse effects (height and weight). Most laboratories currently assess the following endpoints: % emergence, % phytotoxicity, shoot height, plant (shoot) dry weight, and dry root weights. Some registrants have suggested that we only require statistical analysis on <u>one</u> endpoint, such as shoot height. Our experience indicates that no one endpoint is more sensitive than the other all of the time (plants are affected differently by different chemicals). We encourage registrants to supply data on all endpoints. This is an issue for a workshop setting.

18.) Tier III nontarget plant phytotoxicity field studies fall under the "New Paradigm", meaning that they will only be requested in unique* cases. Tier III methods and protocols are topics for workshop discussion.

* Yet to be defined by OPP.

19.) All herbicides, desiccants, and defoliants require a Tier II data set (123-1, 123-2). Exceptions: indoor uses, outdoor domestic (homeowner). (This is a current CFR40, Part 158 issue).

20.) All plant growth regulators require a Tier I data set (122-1, 122-2). Exceptions: indoor uses, outdoor domestic (homeowner). Tier I test effects > 50% for aquatic plants and > 25% for terrestrial plants trigger Tier II data

requirements. (This is a current CFR40, Part 158 issue).

21.) All insecticides, fungicides, and biocides require a Tier I data set (122-1, 122-2). Exceptions: indoor uses, outdoor domestic uses (homeowner). Tier I test effects > 50% for aquatic plants and > 25% for terrestrial plants triggers Tier II data requirements. (This is a current CFR40, Part 158 issue).